ORIGINAL RESEARCH PAPER

Zoology

BIODEGRADATION ASSESSMENT OF OIL CONTAMINATED SOIL AT THANJAVUR DISTRICT

KEY WORDS: oil spreading, length fragment, TLC

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ABSTRACT

Soil samples were collected from different sites of Thanjavur city. Biosurfactants possess both hydrophobic and hydrophilic moieties, they are able to reduce surface tensions and interfacial tension between two fluids at the surface and interfere respectively. We isolated soil bacteria and screened them for biosurfactant activity. The extracts were then separated by thin layer chromatography, oil spreading techniques and Restriction fragment length polymorphism. In oil spread techniques, 20 µl oil was fully degraded and yellow spot was observed by TLC in all the samples. The restriction fragment length shows 2 fragment (petrol bunk and oil mill shop), 4 (mechanical work shop) and 3 (Engine oil mill). The compounds will be further assessed for their activities in oil degradation.

INTRODUCTION

Recently biosurfactants are widely employed the field of bioremediation. Biosurfactant producing organisms were isolated from hydrocarbons contaminated soil samples near petrol bank, automobile shop, oil mill, engine oil spilled soils. Nutrient broth was used while they were identified using for isolation of biosurfactant producing organisms. Biosurfactants synthesized microorganisms structurally a diverse group of surface-active molecules, which are more effective, selective, environment friendly and stable than many synthetic surfactants (4). Oil pollution is caused due to various activities in oil exploration that include geophysical explorations, drilling of wells, pressure control and management of oil and natural gas gushing out from the well, transportation and refining crude etc. The extensive use of petroleum products leads to the contamination (2) and their residues were toxic because they contain large amounts of high molecular mass poly aromatic compounds and heavy metals (5). Enumeration of petroleum degrading microorganisms is important to determine the potential for removal of oil through microbial degradation and to access the amount of oil pollution that has occurred (8). The present study was carried out to reveal the biosurfactant production of *Bacillus subtilis* from oil dropped soils.

MATERIALS AND METHODS

The sample was collected in a sterile polyethylene bag with the help of sterile tea spoon and it was taken to the laboratory immediately and analyzed for the isolation of biosurfactant producing bacteria. The samples were collected from oil contaminates areas such as, petrol bunk, Automobile workshop (Tractor workshop), oil mill, and Engine pump set around Thanjavur district.

Cleaning and Sterilization of Glassware

All the glass were thoroughly cleaned and rinsed with distilled water and dried to ensure that it was free from contamination. Glassware sterilized by autoclaving at 121şC, 15 lbs for 15 minutes.

Screening of Biosurfactant Producing organisms

The isolated colonies were tested for their biosurfactant production by following methods.

Oil Spreading Techniques

To screen the production of bio surfactant producing bacteria the following procedure are as follows,

- 50 ml of distilled water taken in a screw cap bottle and then add 20 µl of petrol, diesel, kerosene oil and 10µl of nutrient broth is added to the bottle.
- The bottle was incubated at 37°C for 10days.
- The results were observed and documented.

Thin layer chromatography

Principle

Thin layer chromatography is based on the principle of separation of the mixed compounds. The separation depends on the relative affinity of compounds towards stationary and mobile phase. Thin layer chromatography is a solid liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents.

Procedure

- A thin line is mark at the bottom of the plate with a pencil to apply the sample spots.
- Then the sample solutions are available on the spots marked on the line at equal distances.
- The spots is dried on the silica gel plate and prepared solvent system chloroform: methanol: Water (65:25:4)3.
- The plate immersed on the solution and removed from the plate after air drying.
- 0.2 g of anthrone 1 ml of sulphuric acid 19 ml of ethanol mixed and the solutions spray on the silica gel plate.
- After drying glycolipid biosurfactant show a yellow color spots.

Restriction fragment length polymorphism

Principle

A restriction digestion takes place by mixing the DNA and the restriction enzymes in a sterile micro tube. It is then incubated at prescribed temperature for a minimum of 3 fours. After the restriction is completed. Agarose gel electrophoresis is performed to separate the digested fragment by sizes.

- It is a difference in the DNA sequences of two homologous chromosomes by identifying the lengths of the restriction fragments after enzymatic digestion.
- The DNA sample is broke into small pieces (digested) by restriction enzymes (RF) that recognize specific sites known as "restriction sites."

RESULT AND DISCUSSION

Oil Spreading Technique

Supernatant of isolated strains were added to the plate containing kerosene oil. It was added to the center of oil layer. The strains was displaced the oil and showing a clear zone. The results were noted down (Fig 1 & Table 1).

Biosurfactant producing organisms was identified by oil spreading techniques and TLC techniques. The culture broth was processed in the nutrient broth medium for the isolation of biosurfactant production by oil spreading techniques and TLC method. Molecular weight of the organisms was determined by restriction fragment length polymorphism method. This study suggests that *Bacillus subtilis* have potential to produce biosurfactant that can be

used for hydrocarbon degrading agent. Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When carbon source is insoluble substrate like hydrocarbon, microorganism facilitates their diffusion into the cell by producing a variety of substances, the biosurfactants. The release of surface active compounds promotes emulsification of the hydrocarbon phase rendering such lipophilic molecules available to the metabolic pathways of microorganism (3).

Thin layer chromatography

Biosurfactant production was screened by thin layer chromatography method. The culture was centrifuged and the supernatant was collected. The samples were used for the identification of rahmnolipid production from the oil contaminated soil. The samples, showed yellow color spots under observation (Table 2 and Fig 2).

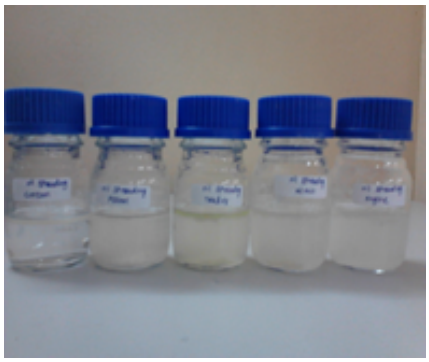


Fig. 1 Oil spreading Test

Fig. 2 Thin Layer Chromatography

TABLE: 1 Oil Spreading Test

S.NO	SOURCES	RESULTS
1.	Petrol bunk soil	20µL of oil fully degraded
2.	Mechanic work shop soil	20µL of oil fully degraded
3.	Oil mill soil	20µL of oil fully degraded
4	Engine oil soil	20µL of oil fully degraded

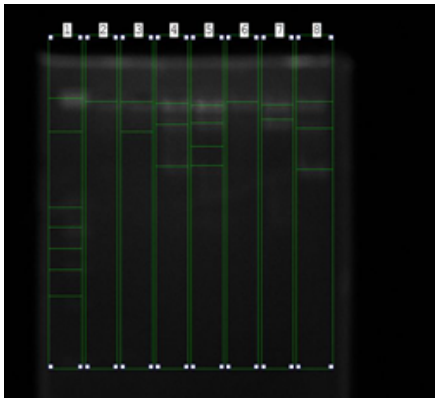
TABLE: 2 Screening of rhamnolipid by thin layer Chromatography

S.NO	SOURCES	RESULTS
1.	Petrol bunk soil	Yellow color spots was observed
2.	Mechanic work shop soil	Yellow color spots was observed
3.	Oil mill soil	Yellow color spots was observed
4	Engine oil soil	Yellow color spots was observed

TABLE: 3 Restriction fragment length polymorphism

S.NO	SOURCES	DIGESTED DNA FRAGMENT BY RFLP
1.	Petrol bunk soil	2
2.	Mechanic work shop soil	4
3.	Oil mill soil	2
4	Engine oil soil	3

In this present study, initially the growth pattern by the isolates on relatively simple hydrocarbon source (engine oil) was studied. Zobell (1965) has reported that growth can be taken as a parameter for microbial utilization of substrate.



M.W. Values	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
Band 1	700.000	694.306	693.172	690.911	687.544	693.172	688.662	694.306
Band 2	650.000		650.000	657.803	659.448		664.744	653.269
Band 3	600.000			648.151	651.155			646.801
Band 4	550.000				648.386			
Band 5	500.000							
Band 6	450.000							
Band 7	400.000							

Fig: 3 Molecular weight analsis by RFLP

Restriction Fragment Length Polymorphism

Restriction fragment length polymorphism is carried out for the identification of the length of the fragment. Hind III restriction enzyme is used for the digestion of the DNA fragment. Electrophoresis is carried out and digested DNA band was observed on the gel (fig 3 & Table 3).

In the present trial study, two different bacterial species that were able to utilize hydrocarbon were isolated and characterized. Similar study has also been reported where the isolation and characterization of a number of bacterial strains have been done which grow at the expense of residues obtained as end products of crude oil processing. Atipan Saimmai (6) reported that *Bacillus subtilis* have higher biosurfactant activity than the *Pseudomonas aeruginosa*. Many species of bacteria are able to decompose and degrade oil. The amount of oil from any spill that is degraded depends upon air and water, temperature, the presence of suitable bacteria and the exact composition of the oil (1).The bacterial isolates viz. *Bacillus subtilis* were screened as hydrocarbon utilizers as they were capable of growing in petrol bunk soil in this present study. Members of the genus *Rhodococcus* are HC degraders (7).

CONCLUSION

From this study, we conclude that all the samples show 20µl of oil degradation in oil spreading test and yellow spots was observed by Thin Layer Chromatographic techniques. The restriction fragment length shows 2 fragment (petrol bunk and oil mill shop), 4 (mechanical work shop) and 3 (Engine oil mill). From this result the further study will be carried out for oil degradation.

REFERENCES

1. Aronstein, B. N., Calvillo, Y. M., & Alexander, M. (1991). Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. Environmental science & technology, 25(10), 1728-1731.
2. Chaillan, F., Le Flčche, A., Bury, E., Phantavong, Y. H., Grimont, P., Saliot, A., & Oudot, J. (2004). Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Research in microbiology, 155(7), 587-595.
3. Karanth, N. G. K., Deo, P. G., & Veenanadig, N. K. (1999). Microbial production of biosurfactants and their importance. Current Science, 116-126.
4. Maneerat, S. (2005). Biosurfactants from marine microorganisms. Songklanakarin J Sci Technol, 27(6), 1263-1272.
5. Mercedes M. Marin, Theo H.M.Smits, Jan B. Van Beilen and Fernando Rojo. 2001. The Alkane hydroxylase gene of Burkholderia cepacia RR10 is under catabolite repression control. J. Bacteriol. 183(14): 4202-4209.
6. Saimmai, A., Rukadee, O., Onlamool, T., Sobhon, V., & Maneerat, S. (2012). Isolation and functional characterization of a biosurfactant produced by a new and

promising strain of Oleomonas sagaranensis AT18. World Journal of Microbiology and Biotechnology, 28(10), 2973-2986.
7. Van Hamme, J. D., Singh, A., & Ward, O. P. (2003). Recent advances in petroleum microbiology. Microbiology and molecular biology reviews, 67(4), 503-549.
8. Walker, J. D., & Colwell, R. R. (1976). Enumeration of petroleum-degrading microorganisms. Appl. Environ. Microbiol., 31(2), 198-207.
9. Zobell, C. E. (1973). Microbial degradation of oil: Present status, problems and perspectives. The microbial degradation of oil pollutants., 3-16.